Social Behavioral Effects of Conditional ACE Knockout in a Subset of Striatal Medium Spiny Neurons in Mice

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Introduction

The angiotensin-converting enzyme (ACE) is part of the renin angiotensin system (RAS) which is responsible for fluid regulation and cardiovascular control, along with having roles in cognition.[1] In the brain, ACE functions to break down endogenous opioid peptides, making them less likely to bind to and activate opioid receptors.[2] There is increasing evidence that angiotensin-converting enzyme (ACE) inhibitors, which are commonly used to treat hypertension, also have positive cognitive effects, but there is still much research to be done.[13,4] One type of ACE inhibitor is the drug trandolapril. Trandolapril is a lipophilic ACE inhibitor, which crosses the blood brain barrier more easily than a hydrophilic ACE inhibitor and is more potent than other ACE inhibitors.[13,14]

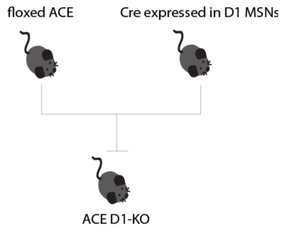
The nucleus accumbens, which is a part of the striatum, has been shown to be a key site for the modulation of opioid peptides on social behavior.[5] D1-type medium spiny neurons (D1-MSNs) in the striatum are GABAergic inhibitory neurons that express the mu-opioid receptor, in addition to ACE.[6] The mu-opioid receptor is involved in response to social stimuli, and is activated by endogenous opioid peptides. The breakdown by ACE of endogenous opioid peptides lowers the amount present, decreasing activation of the mu-opioid receptors. The exact mechanism through which mu-opioid receptor activation enhances social behavior is unknown, but research is ongoing.

Hypothesis: With the injection of the ACE inhibitor trandolapril, the social behavior of wildtype mice will increase, while in mice with ACE genetically deleted from D1-MSNs, the effects of trandolapril will be attenuated.

The injection of an ACE inhibitor such as trandolapril would increase the social behavior in wildtype mice because with the inhibition of ACE, more endogenous opioid peptides would be present in the synapse and free to bind to the mu-opioid receptors, increasing opioid receptor activation. In mice that have an ACE knockout, the effect of trandolapril on social behavior would be attenuated since, without trandolapril's inhibition of ACE, opioid receptor activation during social interaction would stay the same as when trandolapril is not present.

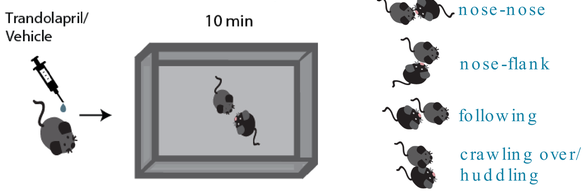
Methods

1. Genetic deletion of ACE from D1 MSNs within the striatum

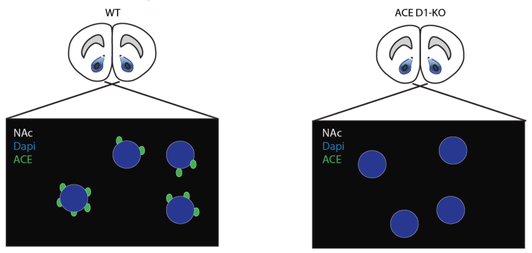


1. Mice expressing floxed *ACE* were crossed with mice that express the Cre enzyme in D1-MSNs resulting in selective genetic deletion of ACE from D1-MSNs
2. At 6 weeks of age, trandolapril or vehicle was administered via intraperitoneal injection to mice 60 minutes prior to pairwise. Time spent interacting socially was then quantified according to behavior type
3. Immunohistochemical analysis of brain slices from the two experimental groups was performed to validate ACE deletion from the striatum of the ACE D1-KO mice. Neurons were co-labelled with the somatic marker DAPI and with an ACE specific antibody. Analysis was performed specifically on anterior brain slices that contain striatal tissue

2. Pairwise Social Interaction Assay



3. Immunohistochemistry



Results

Figure 1. *Social interaction time for a given treatment according to genotype* There was a significant difference in the social interaction time of WT mice after being given vehicle or trandolapril (p -value = 0.0149) There was not a significant difference in the social interaction time of the transgenic ACE D1 -KO mice. The baseline of social interaction time established by the vehicle was significantly different between genotypes (p -value = 0.0037).

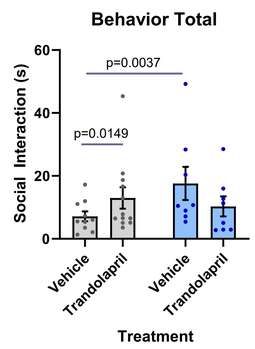


Figure 2 & Figure 3. *Social interaction time spent displaying behavior type according to treatment* There was a significant difference between the WT and transgenic mice in two behavior types when given the vehicle. There was not a significant difference in any of the behavior types between WT and transgenic mice when given trandolapril.

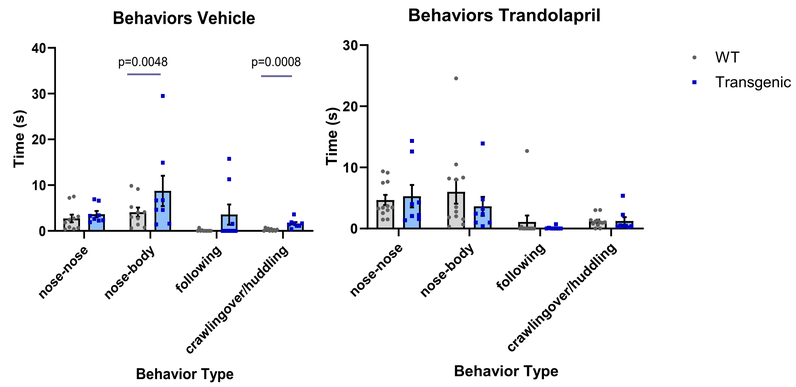
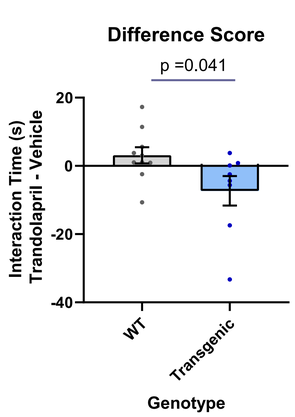
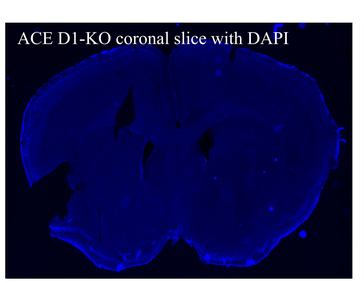
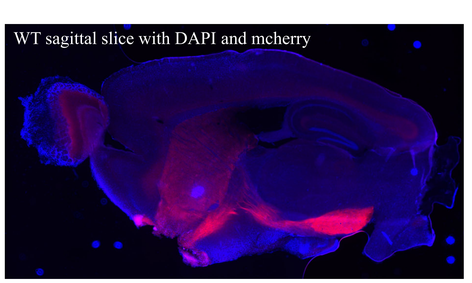


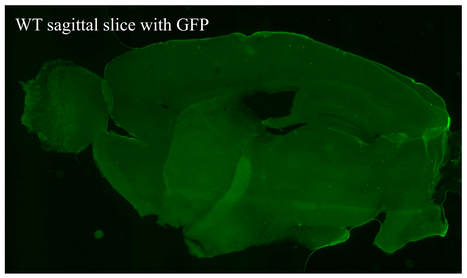
Figure 4. *Difference between interaction times after given treatment according to genotype* There was a significant difference between genotypes in the changes in interaction times after given vehicle or trandolapril (p-value = 0.041). The WT interaction time tended to increase after being given trandolapril while the transgenic interaction time tended to decrease when given trandolapril.



Immunohistochemistry *Failure to validate ACE specific antibody* Brain slices of ACE D1-KO mice (left) had unsuccessful staining of ACE with the ACE specific antibody. Staining of ACE with the ACE specific antibody marked by GFP (green) around the nucleus accumbens should be present based off of prior research. Brain slices of WT mice with mcherry tagged D1-MSNs (right) were also stained with the somatic marker DAPI (blue), mcherry (red) to indicate D1-MSNs and the ACE specific antibody marked by GFP (green). Mcherry and DAPI successfully stained the cells, but the staining by the ACE specific antibody marked by GFP was not present again. In the WT mice, the ACE enzyme is naturally present and has not been deleted, so there should be fluorescence by the GFP. This indicates there is an issue with the ACE antibody or some other factor.







Conclusion

Our hypothesis that the injection of trandolapril would increase the social behavior in wildtype mice, but that the deletion of ACE would allow for the attenuation of trandolapril's effects in the ACE D1-KO mice and the social behavior would remain about the same was incorrect. The results show that while the social behavior of WT mice tended to increase when trandolapril was given, the social behavior of the ACE D1-KO mice tended to decrease. Regardless of treatment there was a significant difference in the social interaction time between genotypes. The ACE D1-KO mice had a higher baseline for social behavior when given vehicle than the WT mice so they may have been naturally more social than the WT mice, but that does not explain the opposite effects of the trandolapril. We also failed to validate a new ACE antibody since there was no staining of ACE in the nucleus accumbens of experimental mice with ACE present or the WT mice with ACE present.

Recommendations

We recommend running the Pairwise Social Behavior assay again with a larger cohort, since in this experiment the lower number of mice led to more uncertainty.

We also recommend looking more into why the knockout of ACE in the ACE D1-KO mice created a greater difference between in their social behavior baseline and the baseline of the WT mice. Additionally, we recommend looking into why the injection of trandolapril in ACE D1-KO mice created an effect on social behavior opposite to that of the WT where the former decreased and the latter increased.

Acknowledgements

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